Kidney International, Vol. 10 (1976) p. 256–263

Soluble immune complexes in sera of patients with nephritis

ROGER D. ROSSEN, MARGARET A. REISBERG, DON B. SINGER, FRANCIS X. SCHLOEDER, WADI N. SUKI, LEIGHTON L. HILL AND GARABED EKNOYAN

Departments of Microbiology and Immunology, Medicine, Pathology and Pediatrics, Baylor College of Medicine and the Laboratories for Immunological Research at the Veterans and the Methodist Hospitals, Houston, Texas

Soluble immune complexes in sera of pateints with nephritis. Binding of radioactively labeled Clq was used to detect soluble antigen-antibody complexes in sera collected at the time of renal biopsy from 104 patients with immunofluorescent findings consistent with immune-complex disease. In comparison with data obtained with sera from 85 healthy donors, significantly elevated Clq binding activity was demonstrated in sera from 22 patients. Clq binding was elevated in all four patients whose dominant histologic finding on bright field microscopy was an intense interstitial mononuclear cell infiltrate. High Clq binding activity was found preferentially in sera from patients who had diffuse rather than focal histologic abnormalities by light microscopy, heavy glomerular deposits of C4 and C3 by immunofluorescence and elevated serum creatinine concentrations. However, there were many patients with similar immunofluorescent and bright field microscopic changes in whom circulating complexes were not detected and there was no correlation between the pattern of glomerular localization of immune complexes and the Clq binding activity of the sera. Serial measurements of Clq binding activity in the sera from three patients over a 90-day interval emphasized that immune complexes may be demonstrated by this technique only intermittently in the sera of some patients with renal biopsy evidence of immune-complex disease. Nevertheless, these observations suggest that the Clq binding test may be a useful tool to monitor disease activity in patients with immunologically mediated renal disease.

Immuns complexes solubles dans le sérum de malades atteints de néphrite. La liaison du Clq marqué radioactif a été utilisée pour déceler les complexes solubles antigène anticorps dans le sérum obtenu au moment de la biopsie rénale chez 104 malades pour lesquels les constations en immuno-fluorescence étaient compatibles avec une maladie à immuns complexes. La comparaison des résultats avec ceux de 85 donneurs en bonne santé a montré une augmentation significative de l'activité de liaison de Clq dans le sérum de 22 malades. La liaison de Clq était élevée chez les quatre malades pour lesquels la constatation histologique principale était une infiltration interstitielle intense faite de cellules mononuclées. Une activité de liaison élevée de Clq a été constatée surtout dans le sérum de malades atteints d'anomalies histologiques diffuses plutôt que focales, avec des dépôts glomérulaires importants de C4 et de C3 en immuno-fluorescence et dont les créatinines sériques étaient élevées. Cependant, chez beaucoup de malades ayant les mêmes modifications histologiques et d'immuno-fluorescence les complexes circulants n'ont pas été décelables et il n'a pas été observé de corrélation entre les modalités de localisation glomérulaire des immuns complexes et l'activité de liaison du Clq. Des mesures répétées de l'activité de liaison de Clq dans le sérum de trois malades sur une période de 90 jours mettent en évidence le fait que les immuns complexes ne sont mis en évidence par cette technique que de façon intermittente dan le sérum de malades dont la biopsie rénale montre des signes de maladie des immuns complexes. Ces observations suggèrent, cependant, que l'épreuve de la liaison de Clq peut être un instrument utile pour surveiller l'activité de l'affection chez des malades atteints de néphropathies à déterminisme immunologique.

Received for publication August 27, 1976;
and in revised form April 13, 1976.

Considerable clinical and experimental evidence suggests that glomerular deposition of antigen-antibody complexes is a major cause of glomerulonephritis [1,2]. Moreover, studies in experimental serum sickness have demonstrated that the degree and extent of glomerular injury are closely related to the quantity of antigen and antibody which deposits in the glomeruli [3,4]. Nevertheless, in man the relationship between circulating immune complexes and the histologic and functional status of the kidney has not been determined primarily due to difficulties associated with the measurement of soluble immune complexes in sera.

Recently, new methods to detect antigen-antibody complexes [5–10] in sera have become available. We have used one of these, the Clq binding method of Nydegger et al [10], to test sera obtained at the time of renal biopsy under the hypothesis that the magnitude of the Clq binding activity in sera may be related to the extent of severity of renal disease.

Methods

Patient and control populations: Collection and storage of sera. Among the renal biopsies submitted for diagnostic evaluation over a period of 27 months, immunofluorescent studies revealed that 111 patients had glomerular deposits of immunoglobulin or complement in a granular pattern consistent with immune-complex disease. Sera were not available from three patients and the clinical records were incomplete in four cases. The remaining 104 patients were included in this study. Clinical records were reviewed to determine the serum creatinine concen-

tration at the time of the biopsy, the endogenous creatinine clearance (ECC), corrected to a body surface area of 1.73 m^2, the quantity of proteinuria and the serum albumin concentration. Sera collected from 85 healthy blood bank donors and stored under the same conditions (−70°C) served as controls. Sera were also collected at frequent intervals from three patients with severe chronic glomerulonephritis hospitalized for treatment with immunosuppressive drugs. None of the patients had clinically apparent infections at the time of the study.

Renal histology. Tissue sections cut at 4 μm from paraffin-blocked renal biopsy specimens were stained with hematoxylin-eosin and periodic acid-Schiff reagents. Initial histologic classification was done immediately after the biopsy specimen was processed. All tissue sections were reviewed independently by one of the authors without knowledge of the previous reading. Based on the pathologists' review of the light microscopic findings, a numerical score from 1 to 3 was assigned to the biopsy: I) within normal limits by bright field microscopy (by immunofluorescence, as noted previously, the glomeruli of these patients also contained granular deposits of immunoglobulin or complement or both); II) focal and/or segmental glomerular abnormalities (some glomeruli were normal by bright field microscopy); and III) all glomeruli abnormal.

Direct immunofluorescence was performed using reagents specific for human-IgG, -IgA, -IgM, -C3 and -C4 [11–13]. Specificity of fluorescein-conjugated antisera to immunoglobulins and complement components was also verified using cryostat sections of glutaraldehyde cross-linked, purified human IgG, IgA, IgM, Fc and Fab fragments of IgG as well as kappa and lambda light chains. Fluorescein-conjugated antisera to C4 and C3 were purchased from Meloy Laboratories (Springfield, Virginia). Their specificity was confirmed by double diffusion in agar gels, immunoelectrophoresis and by membrane immunofluorescence using sheep red blood cells coated with antibody and purified components of human complement. Sheep erythrocytes, antibody and complement components were purchased from Cordis Laboratories, Miami, Florida.

Anti-C4 and anti-C3 did not display immunofluorescence when tested with purified insoluble immunoglobulins or immunoglobulin fragments at concentrations 20 to 60 times those used on renal tissues. Tissue sections were examined by means of a Leitz Orthoplan microscope fitted with Ploem optics.

All sections were evaluated for immunofluorescence by the same observer. Intensity of fluorescence was recorded on a scale of 1+ to 3+. The location of the fluorescence was also recorded for each conjugate, as follows: *focal deposits:* fluorescence present in some but not all glomeruli; *segmental staining:* deposits found in some but not all capillary loops within a glomerulus; *peripheral deposits:* fluorescent staining mainly localized to capillary loops in the periphery of the glomerulus; *mesangial deposits:* fluorescent staining localized chiefly in the stalk areas.

Frequently, patterns of staining in the same glomerulus differed with different antibody conjugates. For example, deposits observed with anti-IgG or anti-IgM might be localized to the periphery of the glomerular capillary loops but deposits delineated by anti-IgA were mainly in the mesangium. To relate serum Clq binding activity to the location of the immunofluorescent material in such cases, we chose as the dominant pattern that which was revealed by the majority of the anti-immunoglobulin and anti-complement reagents. Among tissue sections treated with fluorescein-conjugated anti-IgA, sufficient material (> 3 glomeruli per section) to evaluate localization and intensity of immunofluorescent staining was available in 96 cases. Similarly, the location and intensity of staining with anti-IgG could be evaluated in 95 cases, with anti-IgM in 94 cases, and with anti-C4 and anti-C3 in 85 and 88 cases, respectively.

Estimation of soluble immune complexes in sera. Clq isolated by the method of Yonemasu and Stroud [14] was labeled with ^{125}I by the iodine monochloride method [15]. Fifty nanograms of ^{125}I-Clq was added to 0.2 ml of sera which had previously been heat-inactivated (56° × 30 min) and centrifuged at 7000 × G for 30 min. The binding test was performed in duplicate according to the method of Nydegger et al [10]. This technique takes advanatage of the fact that the reaction product of Clq and many soluble complement-fixing antigen-antibody complexes are insoluble in the presence of 2.5% polyethylene glycol (PEG) whereas unreacted Clq and unaggregated immunoglobulins are soluble in PEG at that concentration [10].

The quantity of Clq bound was estimated by a percentage scale which took as 100% the quantity of Clq precipitated by 10% trichloracetic acid (TCA).

$$\% \text{ Clq bound} = \frac{(\text{CPM } ^{125}\text{I-Clq, experimental}) - (\text{background})}{(\text{CPM } ^{125}\text{I-Clq in 10\% TCA}) - (\text{background})} \times 100.$$

Data were reported as the average percent of Clq bound by duplicate samples. Variation between experiments was monitored by including aliquots of sera from the same two normal donors as negative controls and samples containing 100 and 200 μg of

heat-aggregated IgG (63° × 20 min) as positive controls in each experiment. Mean Clq binding for the first and second of the two normal control sera were, respectively, 3.7 ± 0.3 and 3.6 ± 0.3% (mean ± SEM, ten experiments); 100 and 200 μg of heat-aggregated IgG bound 37.2 ± 1.1 and 58.5 ± 1.5% of Clq, respectively (mean ± SEM, ten experiments). The lower limit of sensitivity of the method, in terms of heat-aggregated IgG was ≤0.5 μg using IgG which had been purified by DEAE cellulose chromotography [11] and aggregated at 63° × 20 min at a concentration of 5 mg/ml. Ninety-five percent of the normal donor sera bound less than 4.7% of the added ^{125}I-Clq. Therefore, 4.7% Clq binding was taken as the upper limit of normal.

Since it is reported that DNA, especially single-stranded DNA (S-DNA), may cause increased Clq binding [10], and since DNA may be present in both normal [17] and patient [16,24] sera, we carried out preliminary experiments to evaluate this potential source of false positives. S-DNA was prepared by heating DNA (calf-thymus, Worthington Biochemicals, Freehold, NJ) to 100°C in a boiling water bath for ten minutes followed by prompt cooling to 0°C in an ice bath. Under the standard conditions of the test, we were unable to show in any of four experiments a significant increase in Clq binding activity in heat-inactivated normal donor sera containing concentrations of added S-DNA or N-DNA ranging from 3 to 200 μg/ml of serum. Sera were tested after heat inactivation in order to abolish competition between ^{125}I-Clq and enodgenous Clq for binding sites on the antigen-antibody complexes and to prevent dissipation of the ^{125}I-Clq in the native Cl complex in the patient's serum [19,21].

Other techniques and methods. C3 concentrations were measured by single radial diffusion in freshly thawed, non—heat-inactivated sera using a commercially available kit (Meloy Laboratories, Springfield, VA). Clq concentrations were also measured by single radial diffusion in 0.75% agarose containing 0.15 M NaCl and 0.01M EDTA buffered at pH 8 with 0.05M Tris glycine using goat anti-Clq lot 231F016 (Kallested Laboratories, Chaska, MN). Serial dilutions of purified Clq [14] were used as standards. The anti-Clq was specific for Clq when tested by immunoelectrophoresis and double diffusion in agarose gels containing 0.01M EDTA against fresh unheated normal human serum with purified Clq as a standard.

Tests for statistical significance were performed by the nonparametric Kruskal-Wallis one-way analysis of variance, the Spearman rank correlation test and the Mann-Whitney U test [18].

Results

Histologic studies of the kidney biopsy specimens demonstrated immune complex-type deposits of immunoglobulin or complement or both in the mesangium or capillaries of some glomeruli from all patients.

Sera from 22 (21%) bound a higher percentage of Clq than did 95% of the healthy controls (Table 1). The highest Clq binding levels were found in sera from patients who had diffuse (group III) as compared to focal (group II) or minimal (group I) histologic evidence of disease as shown by light microscopic examination of the renal biopsy specimen (Fig. 1). The differences in the Clq binding values obtained in these three groups of patients were examined by analysis of variance [18]. Including all observations in patients, the differences in the Clq binding measurements shown in groups I, II and III, in Fig. 1, were marginally different statistically ([H = 5.36, Df = 2, $0.05 < P < 0.10$] analysis of variance [18]). Statistical comparison of the Clq binding activities which exceeded the 95th percentile of the normal range, however, suggested that it was unlikely that the apparently higher values in group III as compared with groups I and II could be attributed to chance alone (U = 6, $P < 0.002$ comparing abnormal Clq binding values in groups I and II with those in group III, Mann-Whitney U test [18]). Clq binding higher than detected in sera from healthy controls was found in 13 patients with idiopathic or poststreptococcal nephritis, in five who had glomerulonephritis associated with a systemic disease and in all four patients in whom the predominant histologic abnormality on bright field microscopy was a diffuse interstitial mononuclear cell infiltrate. In three of

Table 1. Patient Population

	Patients *N*		Clq binding >4.7%	
Glomerulonephritis	100		18	
Idiopathic	72		12	
with nephrotic syndrome		21		4
Rapidly progressive with/without hemorrhagic pneumonitis		7		1
Poststreptococcal	6		1	
Associated with systemic diseases	22		5	
Systemic lupus erythematosus		14		2
Henoch-Schöenlein purpura		2		1
Chronic aggressive hepatitis with hepatitis B antigenemia		1		1
Multiple myeloma		1		1
Malignant melanoma		1		0
Hypertension		1		0
Diabetes mellitus		2		0
Interstitital nephritis	4		4	

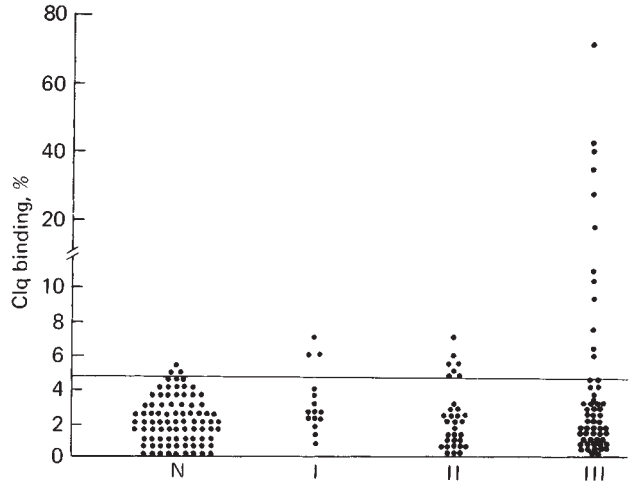

Fig. 1. *Clq binding levels in sera from (N) healthy blood donors and (I) patients with minimal, (II) focal and (III) diffuse disease as judged by histologic examination of renal biopsy specimens by light microscopy.* Ninety-five percent of the healthy donors had serum Clq binding activity <4.7%

these four patients with heavy interstitial infiltrates, bright field microscopy also revealed thickening and fibrosis of Bowman's capsule and synechiae between glomerular tufts and the capsule. All four had granular deposits of C3 in the glomeruli and one had granular deposits of IgM as well by immunofluorescence (Table 1).

Patterns of immune-complex localization in the glomeruli were assessed in relationship to the serum Clq binding activity (Fig. 2). A few patients showed

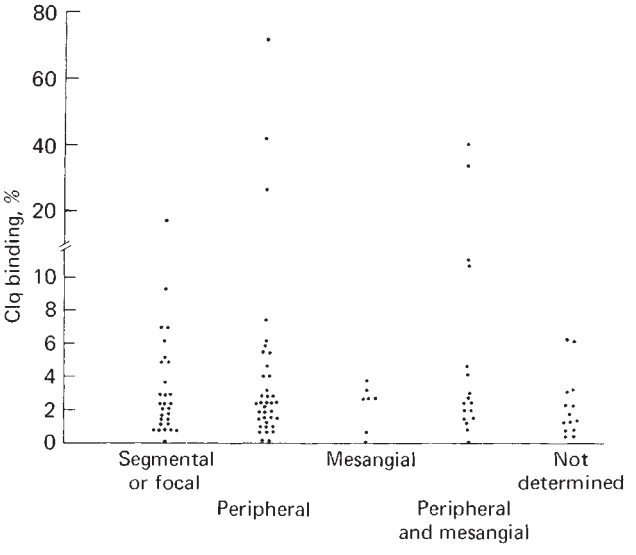

Fig. 2. *Relation of serum Clq binding activity to the dominant pattern of immune-complex localization in the glomeruli, as determined by immunofluorescence.* In 14 cases (last column on the right), the pattern of localization was not determined.

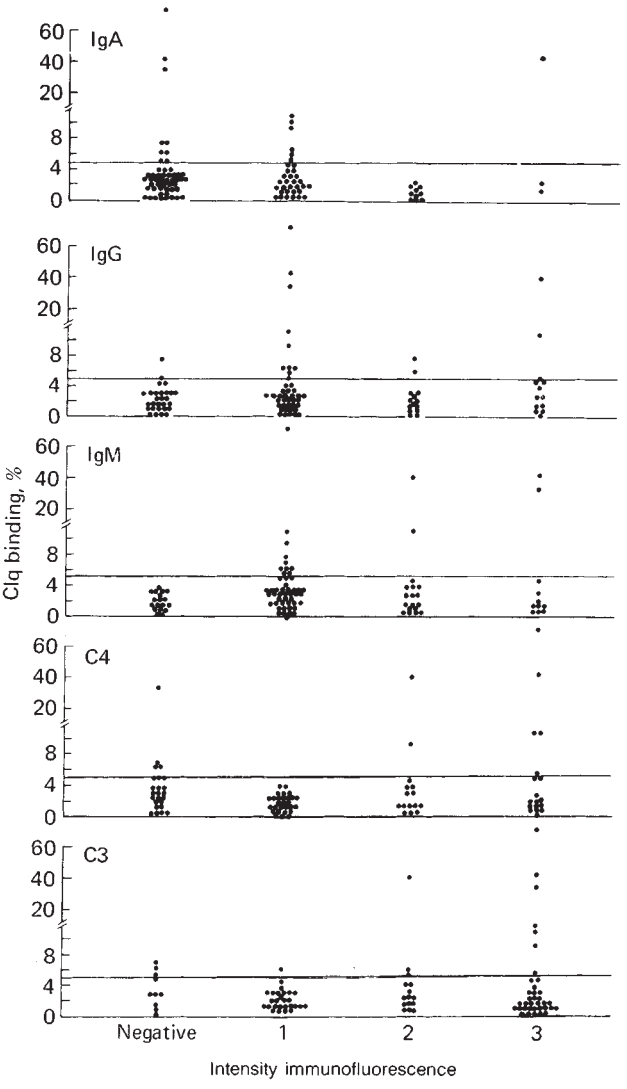

Fig. 3. *Clq binding activity of sera compared to the intensity of immunofluorescence observed with antibody reagents specific for IgA, IgG, IgM, C4 and C3 in the patients' renal biopsy specimens.* High Clq binding activity was associated with intense glomerular immunofluorescence for C3 and possibly for C4 as well.

only mesangial localization of the complexes. None of these had elevated serum Clq binding activity. Among the others, there was no particular immunofluorescent pattern which distinguished the renal biopsy speciments of the 22 patients whose serum Clq binding activity exceeded normal levels. However, patients with the highest serum Clq binding activities frequently had intensely fluorescent deposits of C3 and C4 (Fig. 3). With one exception, Clq binding activity >4.7% was always found in sera of patients whose renal biopsy specimen contained both IgG and IgM in the glomerular immune-complex deposits. Only a few patients had intensely fluorescent deposits

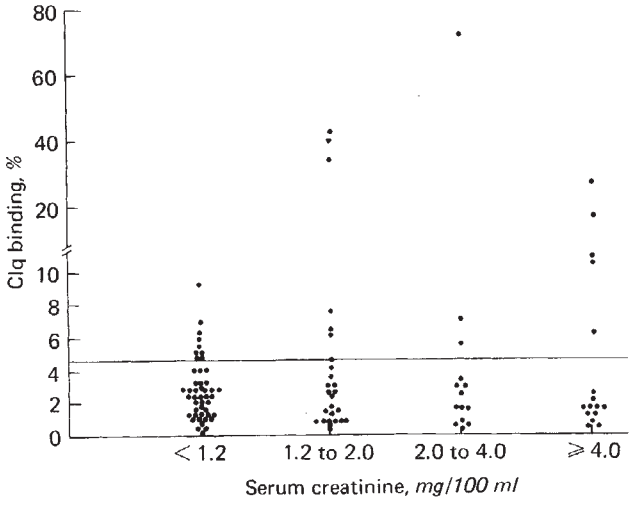

Fig. 4. *Clq binding levels compared to the creatinine concentration in sera from the same patients.* Clq binding of 4.7% indicates the upper limit of the normal range.

of IgA in their glomeruli and serum from only one of these contained high levels of Clq binding activity.

There was no overall correlation between the Clq binding activity of patient sera and their creatinine concentration (analysis of variance comparing the ranks of the Clq binding values for the four groups shown in Fig. 4). However, among patients whose serum Clq binding activity exceeded the 95th percentile value for normal sera, the highest binding activity was found in sera from those with creatinine concentrations greater than 1.2 mg/100 ml (Mann-Whitney U test comparing Clq binding activity in nine patients whose serum creatinine concentrations were <1.2 mg/100 ml with those of 13 patients whose creatinine concentrations exceeded 1.2 mg/100 ml, $U = 15$, $P < 0.002$).

Clq binding activity did not correlate with the serum albumin concentration (data not shown) or the serum concentrations of C3 and Clq (Figs. 5 and 6) (Spearman rank correlation test). Serum Clq concentrations were outside the normal range in only six patients. In 38 patients, C3 concentrations were above those seen in the healthy controls; of these, four had Clq binding activity >4.7%. C3 concentrations in 15 patients were below the normal range and Clq binding activity was >4.7% in six of these.

To evaluate day-to-day fluctuation in the Clq binding activity of sera from patients with immune-complex disease, we studied serial serum samples from three patients, collected at frequent intervals over a 90-day period. In one patient, a 19-yr-old boy receiving intermittent hemodialysis as a result of rapidly progressive glomerulonephritis, the Clq binding activity of sera exceeded 5.0% in only four of 56 samples

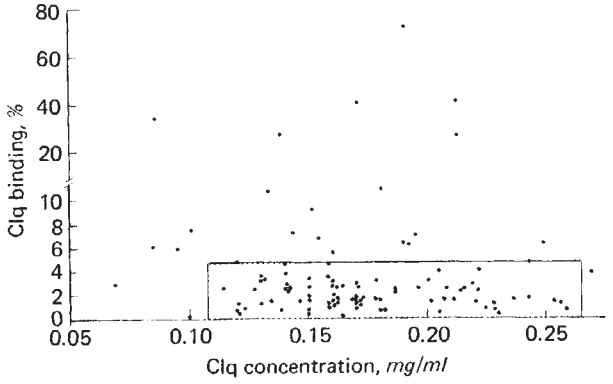

Fig. 5. *Serum Clq concentrations varied independently of the Clq binding activity.* Ninety-five percent confidence limits for the range of normal donor sera are shown within the shaded area.

tested during the 90-day period. In the second patient, (Fig. 7*A*), a 32-yr-old woman who had systemic lupus erythematosus (SLE) with involvement of the central nervous system, kidneys, skin, pleura and pericardium, Clq binding levels exceeded the upper limit of normal (>4.7%) only intermittently. In the third patient (Fig. 7*B*), a 44-yr-old man with rapidly progressive glomerulonephritis, Clq binding activity of the serum remained above the upper limits of normal throughout the period of observation. A decline in the Clq binding activity of his serum, however, paralleled a decrease in proteinuria and a slight decline in serum creatinine concentration observed following the addition of cyclophosphamide to his treatment program.

Discussion

Although the greatest Clq binding activity was found in sera of patients with diffuse cortical disease who, in many cases, had impaired renal function,

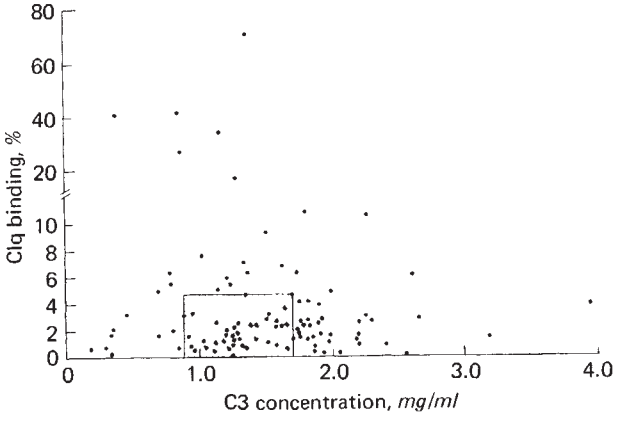

Fig. 6. *Serum C3 concentrations, measured by single radial diffusion, did not correlate with serum Clq binding activity.* Ninety-five percent confidence limits for the range of normal sera are shown within the shaded area.

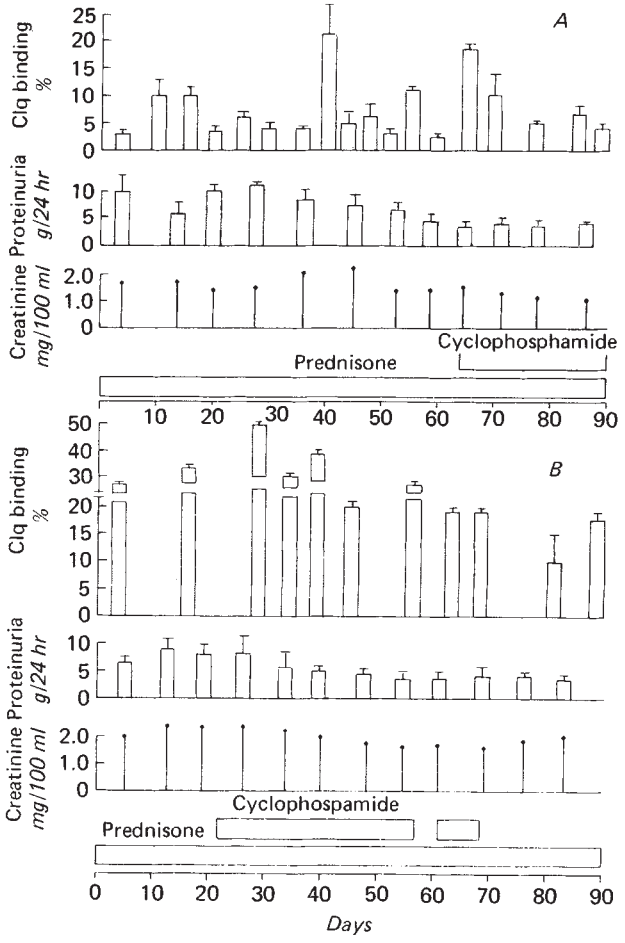

Fig. 7. *A, Serial Clq binding measurements (mean ± SD of at least three determinations) in sera collected at frequent intervals from a 32-yr-old woman with systemic lupus erythematosus.* The serum Clq binding activity is compared to the level of urinary protein loss (mean ± SD of each week's daily 24-hr urine for protein) and the serum creatinine concentration, measured weekly. Times of treatment with prednisone (1 mg/kg) and cyclophosphamide (1 to 3 mg/kg) are shown by *horizontal bars.* (Upper limit of normal Clq binding activity, 4.7%.) *B, Serial Clq binding measurements in sera from a 44-yr-old man with rapidly progressive glomerulonephritis.* Clq binding activity is compared to urinary protein loss, serum creatinine concentration and treatment with prednisone and cyclophosphamide as in *A*.

there were many patients with similar renal histologic and functional characteristics whose sera did not bind abnormally high levels of Clq. Indeed, the incidence of sera which could be considered to contain immune complexes was lower than one would expect based on previous experience with this test in studies of sera from patients with SLE and hepatitis B antigenemia [10]. However, using a test which measures the anti-complementary activity of sera, Johnson, Mowbray and Porter have reported a similar incidence (22.5%) of positive findings for immune complexes in a study of 40 patients who had "glomerulonephritis without fibrinoid necrosis" [8]. Theofilopoulos et al observed that sera from 28 (67%) of 42 patients with immune-complex nephritis contained material which reacted with the C3 receptors of the Raji continuous B-cell line under conditions which can detect 200 to 300 ng of aggregated IgG [9], a level of sensitivity not appreciably greater than that of the Clq binding method (approximately 500 ng of aggregated IgG [10,21]). Sera from patients with glomerulonephritis have recently been examined for immune complexes by a method which measures the ability of the sera to inhibit uptake of radioiodinated heat-aggregated IgG by guinea pig macrophages [7]. Sera from 46 of 78 patients (59%) were reactive in that series including 13 of 18 (72%) with SLE [20]. Direct comparisons of findings of the many tests for soluble immune complexes in the same patient population have not yet been reported. Thus, it is not clear whether these results reflect differences in patient populations, differences in sensitivity of the methods or differences related to the size, immunoglobulin or antigen composition of the immune complexes or the presence of interfering substances.

None of the currently available techniques for measuring soluble immune complexes are ideally satisfactory. All will react with heat-aggregated or denatured IgG. Those which employ Clq as a molecular probe generally require that the sera be heat-inactivated [10,19], a treatment which can make sera anti-complementary and conceivably may dissociate complexes which are held together by low-avidity antibodies [8]. A recently reported modification of the Clq binding test may eliminate the requirement for heat inactivation [21]. False positive reactions are also possible using Clq because of the ability of that molecule to bind to nucleic acids, bacterial products and other materials [10,19,21]. However, the modifications of technique used in the present studies appear to exclude Clq binding by DNA; conceivably, this may have accounted for the comparatively low incidence of positive sera.

Problems with other methods may be equally as great. Recently it has been observed that immune complexes from sera of certain patients with rheumatoid arthritis and vasculitis may potentiate rather than inhibit uptake of ^{125}I-heat-aggregated IgG by guinea pig macrophages [22]. Thus, in using this method one must be prepared to discriminate between two entirely opposite effects of immune complexes in the test system. Similarly, quantitation of the uptake of immune complexes or aggregated IgG in the Raji cell test may be complicated by the fact that C3 or C3b in fresh human sera may promote the

release of cell-bound complexes into the fluid phase [23].

Whichever method has been used to measure immune complexes in sera of patients with nephritis, all studies thus far agree that soluble complexes cannot be found invariably in sera of all patients who have renal histologic evidence of immune-complex disease. The quantitation provided by the Clq binding test has encouraged us to try to relate histologic and functional indices of disease severity to the amount of Clq binding material in the sera. We postulated that severity of disease might reflect both the quantity and the frequency with which the glomeruli are bombarded by soluble immune complexes. The results suggest that, even in a survey conducted at a single time point in the evolution of the disease, high Clq binding activity was detected in sera mainly in patients with the more severe manifestations of renal cortical disease.

Thus, the inability to detect immune complexes in sera from all patients with renal biopsy evidence of immune-complex disease may reflect not so much a lack of sensitivity of the methods but rather differences in the etiologic agents responsible for the glomerular disease, or differences in host-response characteristics such as differences in reticuloendothelial function or antibody-forming capacity which may govern the quantity of antigen-antibody complexes produced and the rate of frequency with which they enter the circulation.

The three cases studied longitudinally illustrate the wide variation to be expected among individuals. The experience with the patient with SLE suggests that complexes may be present only intermittently in the sera in this disease and is reminiscent of previous longitudinal studies which measured DNA, anti-DNA antibody and Clq precipitins in sera of patients with SLE [24,25]. The natural history of chronic glomerulonephritis as a disease with both remissions and exacerbations also suggests that agents or conditions responsible for the formation of immune complexes may be present only intermittently in the circulation [1,2]. Moreover, in experimental nephritis, intermittent injection of antigen can cause renal glomerular disease so long as complexes of the appropriate size are formed and circulate and the capacity of the reticuloendothelial elements outside the kidney to remove and detoxify the complexes is exceeded [26,27].

The assumption that soluble complexes may be present only intermittently in sera from patients with nephritis may partly explain why serum Clq and C3 concentrations were not depressed in patients who had relatively high Clq binding activity. It also provides a rational basis for analyzing separately the data from patients whose serum Clq binding activity was higher than 95% of healthy controls. The correlation of binding activity with severity of disease in these patients suggests that further studies of this and other methods for measuring soluble immune complexes in sera are indicated in patients with various morphologic types of glomerular and interstitial nephritis. Frequent serial measurements may permit more accurate prognosis and serve as guide to therapy.

Similarly, examination of the size distributions as well as immunoglobulin class and the avidity of the antibodies and the molecular characteristics of the antigens incorporated in the circulating immune complexes may permit more precise correlations between the types of complexes found in the sera and the abnormalities which they produce in renal histology. For example, experimental studies which have related the size of circulating immune complexes to their site of localization in the glomerulus suggest that relatively large immune complexes may preferentially seek a subendothelial or mesangial location whereas smaller complexes may be driven selectively to the periphery of the glomerular capillary loops [27].

The present data suggest that this or other means of detecting soluble complexes in patients with various types of renal disease may already be useful in directing attention to conditions which previously were not thought to be mediated by immune complexes. For example, recent observations have suggested that, in some cases, what has been called interstitial nephritis on the basis mainly of light microscopic observations may be an immune-complex disease [28,29]. In this regard, four patients who were included in this series because they had glomerular deposits of immunoglobulin or complement or both by immunofluorescence were found to have interstitial mononuclear cell infiltrates as the dominant lesion by light microscopy and were therefore classified as having interstitial nephritis. The reactivity of their sera with Clq, along with the immunofluorescence observations in these patients, suggests that renal cortical deposition of immune complexes may have been etiologically important in initiating the disease or causing it to persist. Thus, tests for circulating immune complexes may help to identify such patients and discriminate them from those in whom interstitial disease predominantly reflects the influence of cell-mediated immune responses [30].

Acknowledgments

This investigation was supported by Public Health Service grants AM 17555, HL 173690 (i) P-9, 6A,

HL-05435 and RR 00350 and by the Veterans Administration Hospital, Houston, Texas. Dr. Peter Miescher permitted one of us (R. D. Rossen) to visit his laboratory to learn the Clq binding technique and Dr. H. K. Thompson advised us concerning the statical analysis of the data. R. Rickaway, C. Schimbor and A. Solomon provided technical assistance, and E. Bowers and C. Dyess, secretarial assistance.

Reprint requests to Dr. R. D. Rossen, Laboratory for Immunology Research, Veterans Administration Hospital, Houston, Texas 77211, U.S.A.

References

1. COCHRAN CG, KOFFLER D: Immune complex disease in experimental animals and man. *Adv Immunol* 16:186–384, 1973
2. MERRILL JP: Glomerulonephritis. *N Engl J Med* 290:257–266, 313–320, 374–381, 1974
3. WILSON CB, DIXON FJ: Antigen quantitation in experimental immune complex glomerulonephritis: 1. Acute serum sickness. *J Immunol* 105:279–290, 1970
4. WILSON CB, DIXON FJ: Quantitation of acute and chronic serum sickness in the rabbit. *J Exp Med* 134:7S–18S, 1971
5. AGNELLO V, WINCHESTER RJ, KUNKEL HG: Precipitin reactions of the Clq component of complement with aggregated γ-globulin and immune complexes in gel diffusion. *Immunology* 19:909–919, 1970
6. MYLLYLA G: Role of platelets in immune-complex-induced aggregation of human blood platelets. *Ann Med Exp Biol Fenn* 51:22–33, 1973
7. ONYEWOTU II, HOLBOROW EJ, JOHNSON GD: Detection and radio assay of soluble circulating immune complexes using guinea pig peritoneal exudate cells. *Nature* 248:156–159, 1974
8. JOHNSON AH, MOWBRAY JF, PORTER KA: Detection of circulating immune complexes in pathological human sera. *Lancet* 1:762–765, 1975
9. THEOFILOPOULOS AN, WILSON CB, BOKISCH VA, DIXON FJ: Binding of soluble immune complexes to human lymphoblastoid cells: II. Use of Raji cells to detect circulating immune complexes in animals and human sera. *J Exp Med* 140:1230–1244, 1974
10. NYDEGGER UE, LAMBERT PH, GERBER H, MIESCHER PA: Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen: Quantitation by binding to radiolabeled Clq. *J Clin Invest* 54:297–309, 1974
11. REISBERG MA, ROSSEN RD, BUTLER WT: A method for preparing specific fluorescein-conjugated antibody reagents using bentonite immunoadsorbents. *J Immunol* 105:1151–1161, 1970
12. ROSSEN RD, BUTLER WT, REISBERG MA, BROOKS DK, LEACHMAN RD, MILAM JD, MITTAL KK, MONTGOMERY JR, NORA JJ, ROCHELLE DG: Immunofluorescent localization of human immunoglobulin in tissues from cardiac allograft recipients. *J Immunol* 106:171–180, 1971
13. ROSSEN RD, REISBERG MA, SHARP JT, SUKI WN, SCHLOEDER FX, HILL LL, EKNOYAN G: Antiglobulins and glomerulonephritis: Classification of patients by the reactivity of their sera and renal tissue with aggregated and native human IgG. *J Clin Invest* 56:427–437, 1975
14. YONEMASU K, STROUD RM: Clq: Rapid purification method for preparation of monospecific antisera and biochemical studies. *J Immunol* 106:304–313, 1971
15. HELMKAMP RW, GOODLAND RL, BALE WF, SPARR JL, MUTSCHLER LE: High specific activity iodination of globulin with iodine-131 monochloride. *Cancer Res* 20:1495–1500, 1960
16. TAN EM, SCHUR PH, CARR RI, KUNKEL HG: Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J Clin Invest* 45:1732–1740, 1966
17. STEINMAN CR: Free DNA in serum and plasma from normal adults. *J Clin Invest* 56:512–515, 1975
18. SIEGEL S: *Nonparametric Statistics for the Behavioral Sciences.* New York, McGraw Hill Book, Co Inc, 1956
19. SOBEL AT, BOKISCH VA, MULLER-EBERHARD HJ: Clq deviation test for detection of immune complexes, aggregates of IgG and bacterial products in serum. *J Exp Med* 142:139–150, 1975
20. STÜHLINGER WD, VERROUST PJ, MOREL-MAROGER L: Detection of circulating soluble immune complexes in patients with various renal diseases. *Immunology* 30:43–47, 1976
21. ZUBLER RH, LANGE G, LAMBERT PH, MIESCHER PA: Detection of immune complexes in unheated sera by a modified ^{125}I-Clq binding test. *J Immunol* 116:232–235, 1976
22. ONYEWATU I, JOHNSON PM, JOHNSON GD, HOLBOROW EJ: Enhanced uptake by guinea-pig macrophages of radio-iodinated human aggregated immunoglobulin G in the presence of sera from rheumatoid patients with cutaneous vasculitis. *Clin Exp Immunol* 19:267–280, 1975
23. THEOFILOPOULOS AN, DIXON FJ, BOKISCH VA: Binding of soluble immune complexes to human lymphoblastoid cells: I. Characterization of receptors for IgG, Fc and complement and descriptions of the binding mechanism. *J Exp Med* 140:877–894, 1974
24. KOFFLER D, AGNELLO V, WINCHESTER R, KUNKEL HG: The occurrence of single-stranded DNA in the serum of patients with systemic lupus erythematosus and other diseases. *J Clin Invest* 52:198–204, 1973
25. AGNELLO V, KOFFLER AD, EISENBERG JW, WINCHESTER RJ, KUNKEL HG: Clq precipitins in the sera of patients with systemic lupus erythematosus and other hypocomplementemic states: Characterization of high and low molecular weight types. *J Exp Med* 134:228s–241s, 1971
26. DIXON FJ, FELDMAN JD, VAZQUEZ JJ: Experimental glomerulonephritis: The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. *J Exp Med* 113:899–920, 1961
27. GERMUTH FG JR, RODRIGUEZ E: *Immunopathology of the Renal Glomerulus, Immune Complex Deposit and Anti-basement Membrane Disease.* Boston, Little Brown and Co, 1973
28. MCCLUSKEY RT, KLASSEN J: Immunologically mediated glomerular, tubular and interstitial renal disease. *N Engl J Med* 288:564–570, 1973
29. ANDRES GA, MCCLUSKEY RT: Tubular and interstitial renal disease due to immunological mechanisms. *Kidney Int* 7:271–289, 1975
30. ROCKLIN RE, LEWIS EJ, DAVID JR: *In vitro* evidence for cellular hypersensitivity to glomerular basement membrane antigens in human glomerulonephritis. *N Engl J Med* 283:497–501, 1970